Attorney Docket No. ALX-141 PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**



Applicants: Wang, et al.

Serial No.: 08/236,208

Filed: May 2, 1994

For: METHOD FOR THE TREATMENT OF GLOMERULONEPHRITIS

Art Unit: 1806

Examiner: Phillip Gambel, Ph.D.

Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION OF SCOTT A. ROLLINS PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Scott A. Rollins, hereby declare that:

1. I am an inventor of the invention claimed in the above-identified application and have read the office action in the application mailed July 25, 1995.

2. I received a B.S. *summa cum laude* in Biology from the University of Oklahoma, Oklahoma City, OK, in 1986. I received a Ph.D. *summa cum laude* in Immunology in 1990 from the University of Oklahoma, Oklahoma City, OK, where I did my thesis research on molecular and Biochemical analysis of the complement system. I did post-doctoral research for 2 years at the Department of Immunobiology, Yale University School of Medicine, New Haven, CT,

as a Jane Coffin Childs Memorial Fund Postdoctoral Fellow. I am now Director of Complement Biology at Alexion Pharmaceuticals, Inc., New Haven, CT, the assignee of the above-identified application, where I have been on staff since 1992.

3. I have been actively involved in research aimed at the development of pharmaceutical agents comprising C5-specific antibodies and methods for their use for the prevention and treatment of glomerulonephritis.

4. I understand that the Examiner has raised a question regarding the *in vivo* efficacy of complement inhibition using anti-C5 monoclonal antibodies. I believe that the results of the experiments described below show that anti-C5 monoclonal antibodies are highly effective *in vivo*. These experiments were performed under my direction using only routine techniques in accordance with the teachings of the above referenced application.

6. *In vivo* pharmacokinetic analysis of complement inhibition was carried out in the rhesus monkey, which provides an excellent model for the behavior of pharmaceutical agents in humans. The Wurzner et al. N19/8 blocking anti C5 monoclonal antibody (mAb) used in the examples of the above referenced application was used in this analysis.

7. The N19/8 mAb was administered as a single 12.5 mg/kg bolus intravenous injection. This dose was arrived at by routine titration of the concentration of the mAb required to block the complement hemolytic activity of rhesus serum *in vitro* and calculation of a dose expected to approach complete complement

inhibition by extrapolation based upon the conventional assumption (for a drug binding to a serum protein) of a 100 ml/kg volume of distribution in the animal. Only a single such calculation was required to determine this dose, which proved effective *in vivo*.

8. Serial blood samples were drawn pre-injection, and 1, 2, 4, 8, 24, 48, 72, 120, and 144 hours post-injection. These blood samples were allowed to clot at room temperature, centrifuged at 5000xg and the serum decanted and stored at -70°C until testing. The frozen samples were thawed, diluted (10% final) in $GVBS^{++}$, and 100µl/well was added to duplicate wells of a 96 well plate. A standard hemolytic assay was then performed essentially as described in the Materials and Methods section of the above referenced application.

9. As shown in Exhibit A, appended hereto, the pharmacokinetic profile of functional inhibition of rhesus complement hemolytic activity so obtained revealed that serum hemolytic activity was almost completely (greater than 93%) reduced for at least 8 hours post-injection, and returned to pre-treatment levels within 48 hours, with a projected functional T1/2 of approximately 24 hours.

10. As discussed above, these experiments were performed using only standard techniques. The success of this monkey study, involving (inter alia) calculation of dosage and analysis of pharmacokinetic profile, demonstrates the ease with which human dosage and administration parameters can be determined.

11. The *in vivo* data presented in the examples of the above referenced application involve an accepted animal model of human disease in which glomerulonephritis is induced in mice by the injection of foreign proteins that stimulate immune complex deposition in kidney glomeruli ("HAF nephritis"). Additional *in vivo* data are set forth in the "Declaration of Louis A. Matis" submitted herewith. These additional data were obtained using another accepted animal model of human disease, in this case a murine model in which there is a genetic predisposition to the development of a disease state analogous to human systemic lupus erythematosis (SLE). In mice with this genetic predisposition, the course of the inherited disease culminates (as untreated human SLE often does) in severe, and ultimately fatal, immune complex mediated glomerulonephritis ("NZB nephritis").

12. The data obtained in the studies involving both the HAF nephritis and the NZB nephritis animal models consistently indicate that the methods of the invention of the above-referenced application are effective in treating glomerulonephritis *in vivo*. Taken together with the monkey data set forth above, the results of these animal model studies demonstrate that only standard procedures are needed for persons of ordinary skill in the art to effectively use anti-C5 antibodies in the treatment of glomerulonephritis in accordance with the disclosure of the above-referenced application.

13. I declare that all statements made herein of my own knowledge are true and that all statements made on information and

belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Scott A. Rollins, Ph.D.

Signed at New Haven, Connecticut
this 13 day of April, 1996.

## In Vivo Pharmacokinetics of Complement Inhibition by a Blocking Anti-C5 mAb in a Rhesus Monkey

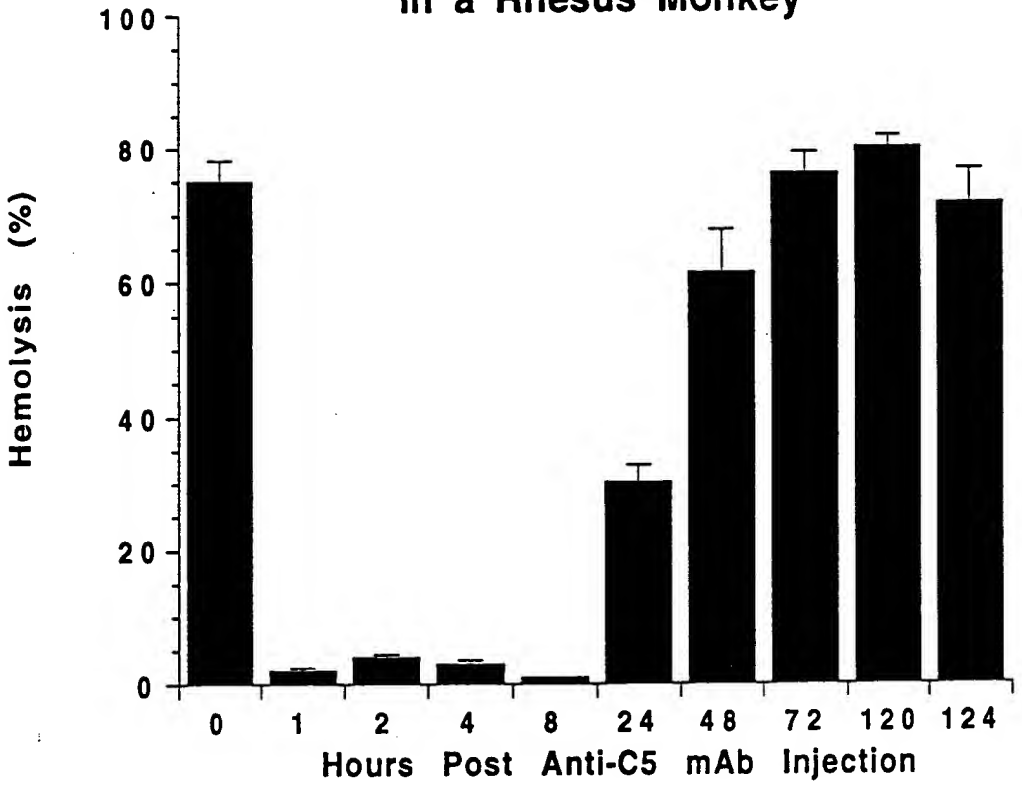


EXHIBIT A